Latitudinal gradient in cortisol concentrations in Canada lynx (*Lynx canadensis*) is not explained by diet

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Latitudinal gradient in cortisol concentrations in Canada lynx 

(*Lynx canadensis*) is not explained by diet

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Latitudinal gradient in cortisol concentrations in Canada lynx (*Lynx canadensis*)

is not explained by diet

C. M. Burstahler, C. V. Terwissen, and J. D. Roth

Abstract

Food limitation is an important stressor for most wildlife, and many specialist consumers will expand their dietary niche to contend with preferred prey limitation. How these dietary responses feed back into stress axis regulation, however, is unknown. If alternative prey does not sufficiently fill the energetic requirements normally satisfied by preferred resources, then long-term glucocorticoid concentrations could be elevated in individuals consuming alternative prey.

We measured cortisol concentrations and stable isotope ratios ($\delta^{13}C$ and $\delta^{15}N$) in hair of Canada lynx (*Lynx canadensis*, Kerr 1792) across their distribution to determine the influence of diet on glucocorticoids, while controlling for harvest location. We calculated the Euclidean distance between lynx and regional snowshoe hare (*Lepus americanus*, Erxleben 1977) stable isotope ratios as an index of diet specialization. We found no relationship between this index and cortisol, suggesting that prey types are interchangeable for lynx in terms of long-term stress axis activation. However, lynx cortisol increased significantly towards the northwestern region of lynx distribution, contrasting with our prediction and highlighting important considerations for future research. This combination of glucocorticoid and diet analyses suggests that dietary plasticity does not necessarily alter an individual’s experience of potential stressors, despite important implications to population- and community-dynamics.

**Keywords:** Canada lynx, *Lynx canadensis*, chronic stress, diet, facultative specialist, geographic gradient
**Introduction**

Phenotypic plasticity may enable species persistence through environmental stressors at the population and community level (Miner et al. 2005), but whether facultative responses of individuals to environmental perturbations influence the stress response is unclear. Decreases in preferred food availability represent one environmental perturbation that can be physiologically stressful to an individual (Fokidis et al. 2012) and often spurs changes in prey choice (Svänback et al. 2011). Food limitation activates the hypothalamic-pituitary-adrenal (HPA) axis, which modulates facultative physiological and behavioural changes including mobilization of energy stores, increase in foraging behaviour, and changes in habitat use (Wingfield et al. 1998). The initial (acute) stress response is advantageous to contend with the immediate threat of food limitation by enhancing the organism’s ability to engage in energetically costly behaviours such as increasing search time or handling time to acquire prey and thus potentially meet minimum energetic requirements. However, failure to attain sufficient food long-term leads to continual activation of the HPA axis (chronic stress), which depletes energy stores and eventually leads to starvation (Wingfield 2003). Chronic stress is generally thought to have other negative consequences to an individual, including reproductive suppression, immunosuppression, and growth suppression (Romero et al. 2009; but see also Boonstra 2013). Thus, facultative use of alternative prey types may curb escalation of HPA activity to a chronic stress response through periods of preferred prey scarcity by meeting minimum energetic requirements and thereby may benefit individuals long-term.

Glucocorticoids such as cortisol, which mobilizes energy stores and modulates the immune system during a stress response, can be measured as an indicator of stress in vertebrates (Busch and Hayward 2009). Acute activation of the HPA axis triggers a negative feedback loop
between signalling from the hypothalamus and glucocorticoid release from the adrenal glands to
deal with the stressor and return to allostasis (Wingfield et al. 1998). Continual activation of the
HPA axis resulting from prolonged exposure to a stressor (chronic stress), diminishes the
feedback effect and leads to accumulation of cortisol systemically and development of
pathologies (Boonstra 2004). Animal tissues with slow turnover rates, such as hair, integrate
circulating cortisol over days to weeks, providing a measure of chronic stress experienced by the
individual in most mammals (Sheriff et al. 2011; but see also Salaberger et al. 2016). With
adequate validation of methods, integrated tissues may provide a window into the chronic stress
physiology of free-living animals (Dantzer et al. 2014).

The decline phase of snowshoe hare (Lepus americanus, Erxleben 1977) cycles marks a
period of severe and prolonged food limitation for Canada lynx (Lynx canadensis, Kerr 1792) in
the boreal forest and is associated with mass mortality of both lynx and hares (O’Donoghue et al.
1997; Krebs 2011). As few lynx live to be >8 years of age (Brand and Keith 1979; Poole 1994;
Slough and Mowat 1996) relative to the 9-10 year length of hare cycles (Krebs 2011), and recent
experience of all lynx approaching peak hare density is one of abundant hares, the threat of hare
scarcity is likely perceived as both novel and prolonged. Yet, Canada lynx are facultative
specialists of snowshoe hare that increase use of alternative prey when snowshoe hares are
limited (O’Donoghue et al. 1998; Burstahler et al. 2016). Use of alternative prey typically
increases in northern regions during snowshoe hare declines (particularly in subadults;
Burstahler et al. 2016) and in the southern parts of their range where hare densities are lower and
more alternative prey are available (Roth et al. 2007; Burstahler 2018). Subadults and peripheral
populations often reflect the margins of a species’ ecological niche but also may demonstrate the
greatest plasticity and potential for persistence in novel and variable environments (Snell-Rood
2013; Pironon et al. 2017), highlighting that the value of facultative responses is context dependent. Thus, increased use of alternative prey by lynx could reflect either successful or unsuccessful mitigation of the stress caused by limitation of preferred prey.

Our objective was to compare diet specialization of lynx across their distribution with cortisol concentrations in hair to identify whether prey choice influences chronic stress experienced by an individual. If alternative prey adequately meet energetic requirements of lynx, then hair cortisol concentrations should be comparable among individuals regardless of dietary specialization or geographic location. Otherwise, if alternative prey do not sufficiently fill the energetic requirements normally satisfied by snowshoe hare, then individuals highly specialized on hares should express lower hair cortisol concentrations than individuals relying heavily on alternative prey and cortisol concentrations should increase towards southern latitudes, where hare densities are typically lower. Combining diet specialization measures with stress hormone analysis will shed light on the utility of alternative prey to specialist consumers and how dietary plasticity relates to individual physiology.

**Methods**

Hair is fed by a blood vessel through the follicle and incorporates systemically circulating molecules during growth, including glucocorticoids and amino acids ( Schwertl et al. 2003; Russel et al. 2012). Once grown, hair is metabolically inert, preserving a record of the animal’s hormones and diet during the period of hair growth (Schwertl et al. 2003). Although local production of cortisol by sebaceous and sweat glands may contribute to cortisol measured in hair of some species (Keckeis et al. 2012; Salaberger et al. 2016), cortisol measured in lynx hair reflects biologically relevant shifts in systemic cortisol during the period of hair growth.
Canada lynx moult biannually over a 6-week period, in April-May and in October-November (Quinn and Parker 1987), thus the cortisol concentrations and stable isotope ratios of winter-trapped samples reflect average autumn conditions for lynx during this period.

Lynx hair was cut from the hind leg of pelts at the North American Fur Auctions (Toronto, ON) and Fur Harvester Auction Inc (North Bay, ON). Pelts were accompanied by the geographic coordinate for the centroid of the furbearer management unit where the animal was harvested. From our collection, we selected lynx specimens with enough tissue to run both stable isotope analysis and hair cortisol analysis (> 1 g). We cut hair samples away from the skin, as close to the base of the hair shaft as possible. Guard hair and underfur may differ isotopically in some animals (Elliott et al. 2017) but are not distinct in lynx winter pelage (paired t-test $\delta^{13}$C: $t_8 = -0.28, P = 0.79$; paired t-test $\delta^{15}$N: $t_8 = -0.35, P = 0.73$) and thus were pooled. To facilitate different washing methods between hormone- and stable isotope- analysis, a small subsample was set aside for stable isotope analysis (roughly 1 mg of hair). Because the stable isotope ratios of consumers depend on those of their prey (Phillips et al. 2014), we also collected white winter guard hair from the haunch of snowshoe hare in 2013 with the assistance of government agencies, scientists, and trappers in Alaska, Yukon Territory, Manitoba, Ontario, and Québec, and we used stable isotope ratios of snowshoe hare hair samples collected from the Northwest Territories in 2001 (Roth et al. 2007). Snowshoe hare stable isotope ratios do not vary from year to year (Roth et al. 2007), but atmospheric changes in $\delta^{13}$C affect comparison of stable isotope ratios across decades and so we corrected the stable isotope ratios of hares from the Northwest Territories to reflect changes in baseline $\delta^{13}$C since 2011 (Long et al. 2005). We tested for regional differences in hare stable isotope ratios using analysis of variance and Tukey’s HSD at a
95% confidence level. Lynx and snowshoe hare hair samples were prepared for stable isotope analysis by washing with soap and water, drying in an oven (60°C), homogenizing to a powder using scissors, and wrapping 0.6 mg subsamples in tin capsules (Elliott et al. 2017). Stable isotope ratios were measured on a continuous-flow stable isotope mass spectrometer at the University of Windsor Chemical Tracers Lab (Windsor, ON).

To prepare lynx hair for hormone analysis, samples were washed with 100% methanol for 20 seconds using a spray bottle to remove surface contaminants, then dried in a fume hood, and cut into 0.5 cm pieces. We then added 1 ml methanol per 0.01 g hair tissue and placed samples on a rotator for 24 hours to extract cortisol (RotoFlex R2000, Argos Technologies, Elgin, IL, USA). We centrifuged samples for 10 minutes at 35 000 rpm then transferred 450 µl of the extracted solution to new tubes and dried the samples in a fume hood. We reconstituted samples in 150 µl enzyme immunoassay buffer solution (0.1 mM sodium phosphate buffer, pH 7.0, containing 9 g of NaCl and 1 g BSA per litre) for a 3-fold concentration, then sonicated samples for 20 seconds in an Elmasonic waterbath (Elma GmbH & Co KG, Germany) prior to hormone analysis. Hair cortisol concentrations were quantified using the enzyme immunoassay procedure described in detail by Terwissen et al. (2013), including critical evaluation of parallelism, precision, accuracy, and extraction efficiency for this method.

Collection locations of our samples covered a large geographic area from Alaska through Québec in 2008 and an additional 23 samples each from Alaska (2009-2010) and Yukon (2006-2007) to capture a mix of prey assemblages and availability. Because snowshoe hares are depleted in both δ13C and δ15N compared to all other prey species and do not vary annually (see Appendix B and D, respectively, in Roth et al. 2007), simple Euclidean distance models can be employed to represent diet specialization on snowshoe hare. We used a two-dimensional
Euclidean distance model measuring the distance in isotope space from individual lynx to the regional mean of snowshoe hare stable isotope ratios after correction for isotopic discrimination. The implicit assumption of a Euclidean distance model is that increasing proximity to the value of snowshoe hares indicates greater consumption of hares, therefore a lynx feeding exclusively on snowshoe hare will have a diet index of 0‰ and lynx feeding increasingly on alternative prey species will have higher value diet indices. As carnivores derive the bulk of their energy intake from the meat of prey, snowshoe hare stable isotope ratios were first corrected for within-animal difference between hair and muscle tissue stable isotope ratios by subtracting 1.5‰ from $\delta^{13}C$ and 0.6‰ from $\delta^{15}N$ based on measurements from European rabbits ($Oryctolagus cuniculus$, Lilljeborg 1873; Neilson et al. 2005). Snowshoe hare stable isotope ratios were then corrected for diet-tissue trophic discrimination measured in Canada lynx ($\Delta^{13}C = 2.4‰$, $\Delta^{15}N = 3.3‰$; Parng et al. 2014). A general linear model was used to compare diet specialization of lynx across locations ($\alpha = 0.05$).

We used multiple regression to measure the influence of diet specialization on lynx cortisol concentration, while controlling for harvest location. Cortisol measurements were log-transformed to fit a normal distribution. We identified multicollinearity among predictors using Pearson correlation coefficients and variance inflation factors (VIF), where a VIF greater than 10 was considered problematic (Quinn and Keough 2002, p. 128). Latitude and longitude were highly correlated predictors ($r = 0.95$). When considered in a multiple regression model of $\log$(cortisol), variance inflation factors exceeded our threshold for latitude (VIF = 11) and longitude (VIF = 11), while diet specialization did not (VIF = 1.0). As such, we combined latitude and longitude using principal component regression into a single axis, PC1, to represent lynx harvest locations where values ranged from northwest Alaska (PC1 = -2) to southeast
Québec (PC1 = 2). PC1 explained 98% (± 1.4 SD) of the variance in harvest location.

Substituting PC1 for latitude and longitude in the multiple regression of log(cortisol) showed considerable improvement to the variance inflation factor for location (VIF = 1.0). PC1 and diet specialization were minimally correlated ($r = 0.10$). All predictor variables were standardized to z-scores that are normally distributed with a mean of zero and a standard deviation of one, so that all coefficients resulting from the model could be compared with equal effect strength. Once all variables were suitably transformed, we conducted a multiple linear regression of lynx diet index and harvest location on cortisol (log-transformed). Finally, we plotted the autocorrelation function of model residuals to confirm independence of observations (Fig. S1)\(^1\) and conducted a post hoc power analysis of the regression to ascertain statistical power to detect a medium ($f^2 \geq 0.15$) or large ($f^2 \geq 0.35$) effect using Cohen’s criteria at $\alpha = 0.05$ (Cohen 1988). All analyses were conducted using R version 3.3.1 (R Development Core Team 2013), including the “car” package for VIF calculations (Fox and Weisberg 2016), the “caret” package for principal components regression (Kuhn 2016), and the “pwr” package for power analysis (Champely 2017).

**Results**

The 117 lynx samples analyzed ranged from the west coast of Alaska to southeastern Québec (Fig. 1). Snowshoe hare stable isotope ratios varied among regions for both $\delta^{13}C$ ($F_{5,66} = 4.3$, $P = 0.0018$) and $\delta^{15}N$ ($F_{5,66} = 7.8$, $P < 0.0001$; Table 1). Mean (±SD) $\delta^{13}C$ measured in lynx was -24.6‰ (0.52) and $\delta^{15}N$ was 5.4‰ (0.97). Diet indices ranged from 0.29‰ to 3.7‰ (mean = 2.0‰, SD = 0.62) and were comparable across harvest locations ($F_{1,115} = 1.0$, $P = 0.31$; Fig. 2a). Hair cortisol concentrations ranged from 0 to 185 ng/g (Fig. 2b). Cortisol concentrations

\(^1\)Supplementary Figure S1 is available with the article through the journal Web site

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increased towards northwestern latitudes \((F_{1,114} = 71, P < 0.0001)\), but were unrelated to diet \((F_{1,114} = 0.27, P = 0.60; \log(\text{cortisol}) = 3.0 + 0.13 \text{ diet} - 0.61 \text{ PC1}, F_{2,114} = 36, P < 0.0001, R^2 = 0.38; \text{Fig. 3})\). Post hoc power analysis indicated an effect size of 0.60, or > 99% power to detect a medium or large effect of diet and location on cortisol.

**Discussion**

Whether individuals were highly specialized consumers of snowshoe hare or used alternative prey, a range of long-term cortisol concentrations were expressed by lynx suggesting that the type of prey consumed does not influence chronic stress in lynx. All regions presented a similar range of diet indices, indicating that we adequately captured a range of environmental conditions prompting niche expansion in some individuals, and post hoc power analysis confirmed our ability to detect any ecologically relevant effect of prey types consumed. Furthermore, we identified a geographic gradient in lynx cortisol concentrations, increasing towards northwestern regions, which contradicts our prediction based on geographic gradients in snowshoe hare density and highlights important considerations for future research.

The use of glucocorticoids to measure physiological responses to and consequences of change in food availability is a relatively new and growing niche of ecology in free-living organisms. Glucocorticoid responses to food availability measured in mammals vary and several mechanisms have been suggested to explain these differences, including the nutritional quality of available foods and behavioural strategies to cope with changing food availability. For example, reduced consumption of fruit leads to elevated fecal glucocorticoids in frugivorous primates despite availability and consumption of alternative foods, suggesting that nutritional quality is important for these species (Pride 2005; Chapman et al. 2007; Foerster et al. 2012). In contrast,
behavioural strategies like feeding on multiple food types (nutrient mixing) and reducing energy expenditure allowed black howler monkeys (*Alouatta caraya*, Humboldt 1812) to fulfill their nutritional requirements during periods of reduced food intake and availability and was not related to fecal glucocorticoids (Martinez-Mota et al. 2016). Addition of new, high quality food types can also enhance energy intake and reduce glucocorticoid concentrations. For example, crop-raiding by olive baboons (*Papio anubis*, Lesson 1827) doubled energy intake and halved fecal glucocorticoids in comparison with the adjacent troop consuming an all-wild diet (Lodge et al. 2013). Finally, Bryan et al. (2014) suggest that the perception of preferred food limitation can affect chronic stress in free-living organisms regardless of prey types consumed. Increasing specialization on salmon (*Oncorhynchus* spp.) by grizzly bears (*Ursus arctos*, Linnaeus 1758) reduced cortisol concentrations measured in hair, but for black bears (*Ursus americanus*, Pallas 1780) in the same region it was the availability of salmon (not the proportion of salmon in the diet) that correlated with cortisol (Bryan et al. 2014). The authors conclude that the direct relationship between prey choice and cortisol concentration in grizzly bears reflects superior nutritional benefit of specialization on salmon, whereas for black bears the perceived social strife of increasing competition for preferred resources is a chronic stressor. The cumulative evidence emphasizes the complexity of physiological and environmental factors influencing long-term glucocorticoid concentrations in free-living organisms and the need for continued effort in measuring multiple covariates alongside glucocorticoids to clarify the relationship between food availability, diet, and chronic stress. The proportion of snowshoe hare in lynx diets measured herein did not relate to cortisol concentrations suggesting that either prey type is adequate, however it remains unknown whether hare scarcity alone (regardless of diet) could drive a chronic stress response in lynx. We strongly recommend future investigations control for prey
availability to disentangle whether perceived scarcity of preferred prey alone influences
glucocorticoids in lynx.

Several biotic or abiotic factors may be driving the increasing geographic gradient in lynx
cortisol concentrations observed here, including the physiology of moult, differences in climate
(Boonstra 2004), prey availability (Fokidis et al. 2012), density of competitors (Ritchie and
Johnson 2009), or anthropogenic influences (Dantzer et al. 2014). First, moulting is regulated
through the HPA-axis, which is triggered by some environmental cue (e.g. photoperiod) and thus
could be expressed differently across an animal’s distribution. For example, the early and rapid
onset of harsh winter climates towards northern latitudes may require mammals to grow
insulating winter pelage denser and faster, spurring greater upregulation of the HPA-axis and
possibly resulting in higher cortisol incorporation. Furthermore, mobilization of energy stores to
maintain body mass throughout the winter is also regulated through the HPA-axis (Boonstra
2004); thus, it is possible that timing of moult is synchronous across the distribution and the
elevated cortisol concentrations of northwestern lynx reflects longer exposure to cold
temperatures. In addition, environmental stressors can both upregulate and suppress stress axis
activation (Dantzer et al. 2014). For example, adult Magellanic penguins (*Spheniscus
magellanicus*, Forster 1781) occupying areas that were regularly visited by tourists showed
diminished glucocorticoid release in response to human visitation than those that were not
habituated to human visitation; yet, all penguins responded similarly to the novel stressor of
capture and restraint, indicating that HPA-axis activity was not compromised in either group
(Walker et al. 2006). Thus, the consistently lower cortisol concentrations measured in
southeastern lynx may reflect habituation to less favourable environmental conditions and
downregulation of HPA activation due to the regularity of noxious stimuli such as low and
unpredictable snowshoe hare availability, higher densities of competitors, and many anthropogenic disturbances imposed by high human densities (Murray et al. 2008). In contrast, lynx in the northwestern region of their distribution occupy relatively undisturbed habitat by comparison (Murray et al. 2008). Larger tracts of undisturbed forest and strong cyclicity of snowshoe hares mean that most individuals will be accustomed to living with abundant food and fewer perturbations. Under these circumstances, stressors such as snowshoe hare decline, a severe storm, or proximity to human disturbance would undoubtedly be perceived as novel, life-threatening stimuli to most individuals. Together, the growing repertoire of field studies highlighting variable responses to common stressors suggests both species- and context-dependent nuances to stress physiology and a current limitation to broad application and interpretation of cortisol concentrations without thorough control of ecological covariates.

Hair cortisol analysis is increasingly used to address ecological questions in free-living organisms, but the relative novelty of this method comes with associated unknowns and limitations. Ambiguity surrounding the physiology of moult at continental scales is a major limitation for studies using these increasingly available endocrine tools and warrants critical evaluation to facilitate application to free-living organisms. In addition, cortisol data are inherently noisy because many intrinsic factors can affect glucocorticoids including sex, reproductive condition, age, and individual differences, each of which can reduce our ability to detect physiological responses when not adequately accounted for (Dantzer et al. 2014). This study benefits from the validation of an enzyme immunoassay specific to Canada lynx and evidence that hair cortisol does not appear to vary with age or sex in lynx (Terwissen et al. 2013). However, uncertainty remains about the mechanisms of cortisol incorporation into the hair shaft. Blood supply through the follicle is thought to be the primary source of glucocorticoid
incorporation (Sheriff et al. 2011), yet local production of cortisol can influence measurement in some instances (Sharpley et al. 2009; Keckeis et al. 2012). For example, mechanical stress to the skin (brushing) increased cortisol concentrations measured in sheep hair (Salaberger et al. 2016) and could bias results if an animal had a pre-existing condition causing irritation near the sampling site. Wild-harvested lynx in North America are trapped using soft-catch leg-hold restraining traps that target the forelimbs or killing traps that target the neck and shoulders, both of which must be checked daily (Fournier and Canac-Marquis 2017). Thus, our measurements from the hind leg are unlikely to be affected by mechanical stress of the trapping process. The use of hair as an integrated measure of long-term cortisol concentrations is a recent addition to ecological studies that holds great promise for extending our understanding of how environmental changes affect animal populations, but we echo the cautionary note that its application requires careful validation of methods, consideration of covariates, and tentative discussion of implications (Busch and Hayward 2009; Dantzer et al. 2014).

The occurrence and function of chronic stress in free-living animals appears to be species-specific and related to the ecological and evolutionary context of those organisms. For example, high predation risk elicits a strong chronic stress response in snowshoe hare but not in elk (*Cervus canadensis*, Erxleben 1777), likely due to differences in the frequency of predation risk (periodic vs. constant) and the lifespan of the animal (Boonstra 2013). Given that few lynx in their core range will experience multiple declines in snowshoe hare abundance and the majority of their life experience leading up to the peak of a cycle is one of abundant hares, it remains possible that snowshoe hare scarcity alone (regardless of diet) could elevate glucocorticoids and contribute to reduced reproductive output during this time (Brand and Keith 1979). In contrast, lynx populations at the southern extent of their distribution experience
dampened fluctuations in hare abundance (Murray et al. 2008), eat more generalized diets (Roth et al. 2007), and experience lower long-term cortisol concentrations than conspecifics to the north. Thus, in addition to the short-term mechanisms discussed above, geographic differences in glucocorticoids could be explained by long-term evolutionary adaptations to different environmental conditions. Determining whether snowshoe hare scarcity alone influences glucocorticoids in lynx is an important next step, as this could have important implications to population dynamics during cyclic declines in northern regions and irruptive fluctuations in southern regions. Our goal herein was simply to associate a physiological state with an ecological phenomenon in a free-living organism, but the collective data suggest that we have not yet learned enough about the generality of chronic stress patterns in nature to draw strong conclusions or implications from observational studies. We recommend future investigations control for as many ecological covariates as possible and, in particular, consider prey availability alongside prey choice to disentangle whether perceived scarcity of preferred prey, in addition to prey types consumed, influences chronic stress.

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Table 1. Regional differences in δ13C and δ15N of snowshoe hare (*Lepus americanus*, Erxleben 1977) winter guard hair.

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a,b,c Means with the same letter do not differ significantly.

* δ13C values from the Northwest Territories were corrected for changes in atmospheric carbon since 2001 (Long et al. 2005).
Figures

**Figure 1:** Harvest locations of Canada lynx (*Lynx canadensis*, Kerr 1792) in Canada (white) and Alaska, USA (grey). Points represent the centroid of the jurisdictional harvest management unit from which lynx were trapped. Map sources: Esri, HERE, Garmin, FAO, NOAA, USGS, © OpenStreetMap contributors, and the GIS User Community.

**Figure 2:** Effects of latitude on (a) diet index and (b) cortisol concentrations (ng/g) of Canada lynx (*Lynx canadensis*, Kerr 1792). Diet index values closer to zero indicate lynx whose stable isotope ratios overlap with mean regional snowshoe hare stable isotope ratios (after correction for trophic discrimination), and thus consumed a highly specialized diet. Higher diet index values indicate increased consumption of alternative prey. One extreme cortisol concentration value (185 ng/g) has been omitted from the figure but was included in the analysis.

**Figure 3:** Effects of (a) diet index and (b) harvest location on cortisol concentrations (ng/g, log-transformed) in Canada lynx (*Lynx canadensis*, Kerr 1792) hair. Diet index values closer to zero indicate lynx whose stable isotope ratios overlap with mean regional snowshoe hare stable isotope ratios (after correction for trophic discrimination), and thus consumed a highly specialized diet. Higher diet index values indicate increasing consumption of alternative prey species by lynx. Harvest location is presented as the principal components regression (PC1) between latitude and longitude of the centroid of the jurisdictional harvest management unit from which lynx were trapped.
Figure 1

197x83mm (300 x 300 DPI)
Figure 2

Diet index (%) vs. Latitude

Hair cortisol (ng/g) vs. Latitude

212x132mm (300 x 300 DPI)